Photosensitization of leishmania with URO and PC-14

Photosensitizers (PS) can be delivered endogenously with delta-aminolevulinate (ALA) and exogenously with phthalocyanines (PC). DT mutants at $5x10^7$ cells/ml were exposed to 1mM ALA and 0.1µg/ml PC-14 in Hank's Balanced Salt Solution (pH=7.4) + 0.01% BSA (HBSS+BSA) for ~48hrs at 25 ^oC in the dark to induce double photosensitization. The cells were washed by centrifugation at 3,500 x g for 5 min at 4 ^oC thrice in HBSS to remove extracellular ALA and PC-14. To optimize the loading efficiency, the cells were incubated in 0.01% BSA-HBSS in the dark overnight. No adverse effect produced when the cells in the dark even if they were exposed to PS. Before use, resuspend the cells in HBSS-BSA at a cells density 10^8 cells/ml.

Illumination of photosensitized leishmania

Suspensions of sensitized leishmania were placed at 10^8/ml/well in 12-well tissue culture plate. Illuminated with long-wave UV lamp (365 nm) from the top (needed to remove the lid of the plate) for 20 min and red light (620~630 nm) from the bottom of the plate for 10 min to photodynamically excite URO and PC-14, respectively. Under the illumination of the cells, carefully prevent the high temperature from ambient light source.

Endocytosis of photo-inactivated leishmania in DC2.4 cells

After specific light illumination to excite URO and PC-14, leishmania were noted to remain structurally intact. Although the cells were unable to grow and perish eventually, they were able to infect DC2.4 cells as well as normal leishmania. DC2.4 cells were infected with photo-inactivated leishmania at host-parasite ratio 1:10. Mix a suspension of 10^7 cells/ml/well DC2.4 cells and 10^8 cells/ml/well photo-inactivated leishmania in 12-well tissue culture plate, grown in RPMI-1640 supplemented with 20% HIFBS for----------?hrs?